Brilliant Green Agar Modified ISO

Cat. 1143

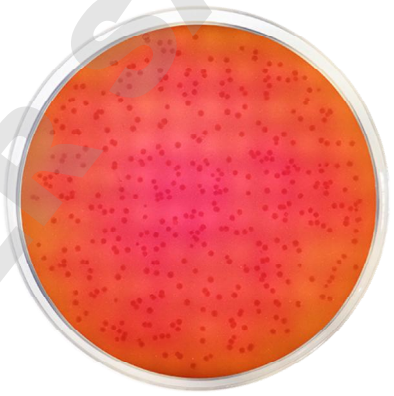
For the selective isolation of Salmonella

Practical information

Aplications	Categories
Detection	Salmonella

Industry: Water / Food

Regulations: ISO 19250 / ISO 6579



Principles and uses

Brilliant Green Agar Modified is a selective medium for the isolation of Salmonella, except for S.typhy, from water,food and animal feed stuffs.

Brilliant Green Agar Modified inhibits the growth of Pseudomonas aeruginosa and partially inhibits the growth of Proteus spp. which may be similar in appearance to Salmonella.

Salmonella can be present in small numbers and is often accompained by considerably larger numbers of other Enterobacteriaceae or bacteria of other families. A preenrichment stage is used to permit the detection of low numbers of Salmonella or injured Salmonella.

Beef extract, Casein peptone and Meat peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Lactose and Sucrose are the fermentable carbohydrate providing carbon and energy. Phenol red is the pH indicato. Brilliant green inhibits Gram-positive and most Gram-negative bacteria, except Salmonella. If the medium overheats, brilliant green may lose its properties. Bacteriological agar is the solidifying agent.

The ISO normative 6579 recommends the Brilliant Green Agar as a second selective medium.

Formula in g/L

Bacteriological agar	15	Beef extract	5
Brilliant green	0,005	Casein peptone	5
Disodium phosphate	1	Lactose	10
Meat peptone	5	Phenol red	0,09
Sucrose	10	Yeast extract	3
Monosodium Phosphate	0,6		

Preparation

Suspend 54,7 grams of the dehydrated medium in one liter of distilled water and leave for 15 minutes. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. Sterilize in autoclave at 115 ºC for 15 minutes. Dispense into appropriate containers.

Instructions for use

* For detection of Salmonella spp. in food, animal feed, animal faeces, and environmental samples:

- Preenrichment in non-selective liquid medium:
Inoculate the Buffered Peptone Water (Cat. 1402) with the sample or dilutions, and incubate at 34-38 ºC for 18 h.
- Enrichment in/on selective media:

Inoculate, with the culture obtained in the pre-enrichment stage, the Rappaport Soy Broth (Vassiliadis)(Cat. 1174) or the Modified Semisolid Rappaport Vassiliadis medium (MSRV) (Cat. 1376), and the Tetrathionate Broth (Muller-Kauffmann) (Cat. 1173).
The Rappaport Soy Broth and the Modified Semisolid Rappaport medium are incubated at 41,5 ºC for 24 h, and the Tetrathionate Broth at 37 ºC for 24 h.
- Plating out on selective solid media:
From the selective enriched cultures, inoculate two selective isolation agar; XLD agar (Cat. 1274) and any other selective medium complementary to XLD agar (Salmonella Chromogenic Agar (Cat. 1122), Brilliant Green Agar (Cat. 1143), Bismuth Sulfite Agar (Cat. 1011), DCLS Agar(Cat. 1045), Desoxycholate Citrate Agar (Cat. 1067), Hektoen Enteric Agar (Cat. 1030), Salmonella Shigella Agar(Cat. 1064) and XLT4 Agar (Cat. 1159)).
Incubate the XLD plates inverted at 37 ºC for 24±3 h.
Incubate the second selective medium in accordance with the manufacturer's instructions.
- Confirmation:
Subculture colonies of presumptive Salmonella and confirm their identity by biochemicals and serological tests.

* For detection of Salmonella spp. in water samples:

- Preenrichment in non-selective medium:
Inoculate the Buffered Peptone Water (Cat. 1402) with the sample or dilutions, and incubate at 36±2 ºC for 18±2 h.
- Enrichment in selective media:
Inoculate, with the culture obtained in the pre-enrichment stage, the Rappaport Soy Broth (Vassiliadis)(Cat. 1174) and the Tetrathionate Broth (Muller-Kauffmann) (Cat. 1173).
The Rappaport Soy Broth is incubated at 41,5±1 ºC and the Tetrathionate Broth at 37±1 ºC, both of them for 24±3 hours.
- Plating out on selective solid media:
From the selective enriched cultures, inoculate two selective isolation agar; XLD agar (Cat. 1274) and any other selective medium complementary to XLD agar (For instance, Brilliant Green Agar (Cat. 1143) or Bismuth Sulfite Agar (Cat. 1011))
Incubate the XLD plates inverted at 36±2 ºC for 24±3 hours.
Incubate the second selective medium in accordance with the manufacturer's instructions.
- Confirmation:
Subculture colonies of presumptive Salmonella and confirm their identity by biochemicals and serological tests.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
Sin restos	Fine powder	Red	Red	6,9 ± 0,2

Microbiological test

Incubation conditions: (37±1 ºC / 24±3 h)
Inoculation conditions: Productivity qualitative (10^3-10^4 CFU) / Selectivity (10^4-10^6 CFU) / Specificity (10^3-10^4 CFU)

Microrganisms	Specification	Characteristic reaction
Salmonella enteritidis ATCC 13076	Good growth	Red colonies, surrounded by a diffused red halo
Salmonella typhimurium ATCC 14028	Good growth	Red colonies, surrounded by a diffused red halo
Salmonella typhi ATCC 19430	Inhibited-Moderated growth	Red colonies
Escherichia coli ATCC 25922	Inhibited-Moderated growth	Yellow green colonies
Staphylococcus aureus ATCC 25923	Inhibited growth	

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

UNE-EN-ISO 6579 Microbiology of food and animal feeding stuffs -- Horizontal method for the detection of Salmonella spp.
ISO 19250 Water quality — Detection of Salmonella spp.